PB86-213709

Humoral and Cell-Mediated Immune Status of Mice Exposed to 1,1,2-Trichloroethane

Medical Coll. of Virginia, Richmond

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1985

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before comple

1. REPORT NO. EPA/600/J-85/416	2.
3. PB86-213709	
4. TITLE AND SUBTITLE HUMORAL AND CELL-MEDIATED IMMUNE STATUS OF MICE EXPOSED TO 1,1,2-TRICHLOROETHANE (Journal Version)	5. REPORT DATE 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Virginia M. Sanders, Kimber L. White, Jr., George M. Shopp, Jr., and Albert Munson	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Medical College of Virginia Richmond, VA 23298-0001	10. PROGRAM ELEMENT NO. CBMC1A
	11. CONTRACT/GRANT NO. CR806481
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory (HERL) Office of Research and Development (ORD) U.S. Environmental Protection Agency (USEPA) Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED Journal Article
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES
P.O. Lyman Condie (for Campbell) TOTB/TMD/HERL/ORD/USEPA (CI)
Citation: Drug and Chemical Toxicology, 8(5), 357-372. October 1985

16. ABSTRACT

The purpose of this study was to assess the immunological effects of 1,1,2-trichloroethane (TCE) on random-bred CD-1 mice following 14 and 90 days of oral exposure. A toxicological evaluation conducted at the same time revealed the target organs to be tne liver of both sexes and the erythroid elements of the females. The 14-day immunological range-finding study in males exposed to doses 1/10 and 1/100 the LD50 (38 and 3.8 mg/kg) revealed no alterations in either humoral or cell-mediated immune status. Following 90 days of exposure in the drinking water (4.4., 46, and 305 mg/kg for males and 3.9, 44, and 384 mg/kg for females), a more detailed series of immunological parameters was assessed. Cell-mediated immunity was unaltered in both sexes, while humoral immune status was depressed in both sexes, particularly when determined by hemagglutination titers. Macrophage function was depressed only in the males as indicated by the ability of thioglycolate-recruited peritoneal exudate cells (PEC) to phagocytize sheep erythrocytes (sRBC).

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 17
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA/600/J-85/416
JOURNAL ARTICLE

DRUG AND CHEMICAL TOXICOLOGY, 8(5), 357-372

HUMORAL AND CELL-MEDIATED IMMUNE STATUS OF MICE EXPOSED TO 1,1,2-TRICHLOROETHANE

Virginia M. Sanders, Kimber L. White, Jr.,
George M. Shopp, Jr., and Albert E. Munson
Department of Pharmacology and Toxicology
Medical College of Virginia/Virginia Commonwealth University
Richmond, VA 23298-0001

ABSTRACT

The purpose of this study was to assess the immunological effects of 1,1,2-trichloroethane (TCE) on random-bred CD-1 mice following 14 and 90 days of oral exposure. A toxicological evaluation conducted at the same time revealed the target organs to be the liver of both sexes and the erythroid elements of the females. The 14-day immunological range-finding study in males exposed to doses 1/10 and 1/100 the LD50 (38 and 3.8 mg/kg) revealed no alterations in either humoral or cell-mediated immune status. Following 90 days of exposure in the drinking water (4.4., 46, and 305 mg/kg for males and 3.9, 44, and 384 mg/kg for females), a more detailed series of immunological parameters was assessed. Cell-mediated immunity was unaltered in both sexes, while humoral immune status was depressed in both sexes, particularly when determined by hemagglutination titers. Macrophage function was depressed only in the males as indicated by the ability of thioglycolate-recruited peritoneal exudate cells (PEC) to phagocytize sheep erythrocytes (sRBC).

INTRODUCTION

Evaluation of the status of the immune system is assuming a significant role in the assessment of compounds. In the previous paper[1], many of the general toxicological parameters were

Copyright © 1985 by Marcel Dekker, Inc. 0148-0545/85/0805-0357$3.50/0

described for CD-1 male and female mice exposed to TCE. The targets of toxicity were the liver of both sexes and the erythroid elements of females.

This manuscript addresses the effects of TCE on the immune system. This work represents the first immunotoxicological evaluation of this compound and demonstrates the feasibility of such an investigation using a random-bred mouse. Although the preferred animal for toxicological investigations is the random-bred rat, we have chosen the random-bred mouse, since immunological assays have been standardized in the CD-1 mouse in our laboratory.

METHODS

Details of the methods for the immunotoxicological assays and historical controls have been fully described in the first article of this volume[2].

Exposure of Animals

Complete details concerning the housing of random-bred CD-1 mice and their exposure to TCE were given in the preceding paper[1]. The actual doses of TCE consumed as time-weighted averages of the three concentrations in the drinking water were also previously described[1]. In the 14-day gavage study, animals were sacrificed the day after the final gavage. For the drinking water study, animals were maintained on TCE until sacrificed.

Statistical Evaluation

If a one-way analysis of variance of the means showed treatment effects, a Duncan's Multiple Range Test was performed[3]. Values which differ from vehicle control at $p < 0.05$ were considered significant. Values represent the mean ± SE.

RESULTS

No alterations in humoral (Table 1) or cell-mediated (Table 2) immune status were observed in male CD-1 mice exposed to TCE for 14 days at doses 1/10 and 1/100 the LD50 (38 and 3.8 mg/kg). Therefore, the 90-day drinking water study using male and female mice was designed to deliver doses equivalent to, and higher than, those delivered in the 14-day range-finding study (4.4, 46 and 305 mg/kg for males and 3.9, 44, and 384 mg/kg for females).

TABLE 1
Humoral Immune Response to sRBC in Male CD-1 Mice Exposed to Gavage for 14 Days to TCE[a]

Exposure Group	Body Wt. (g)	Spleen Wt. mg	Spleen Wt. % Body Wt.	AFC[b]/10^6 Spleen Cells	AFC/Spleen ($\times 10^{-5}$)
Vehicle[c]	30.1 ± 1.0	207 ± 8	0.69 ± 0.02	2687 ± 311	4.46 ± 0.62
3.8 mg/kg	29.3 ± 0.8	184 ± 11	0.63 ± 0.04	2002 ± 196	3.44 ± 0.53
38 mg/kg	28.0 ± 0.8	172 ± 5*	0.62 ± 0.03	2089 ± 170	3.08 ± 0.38

[a]Values represent mean ± SE derived from 11-12 mice per group.
[b]AFC = antibody forming cells
[c]Vehicle = 10% emulphor
*Significantly different from vehicle at $p < 0.05$.

TABLE 2
Cell-Mediated Immune Response to sRBC in Male CD-1 Mice Exposed by Gavage for 14 Days to TCE[a]

Exposure Group	Stimulation Index
Vehicle[b]	3.88 ± 0.36
3.8 mg/kg	5.00 ± 0.50
38 mg/kg	4.16 ± 0.45

[a]Values represent mean ± SE derived from 12 mice per group. The unsensitized value was 1.47.
[b]Vehicle = 10% emulphor.

Following a 90 day exposure, humoral immune status was evaluated by enumeration of IgM antibody forming cells (AFC) against sRBC, measurement of hemagglutination titers, and evaluation of spleen lymphocyte responsiveness to lipopolysaccharide (LPS).

Table 3 summarizes the IgM AFC data for day 4 (peak day) and day 5 following sRBC immunization. Male mice exhibited no change from control on the peak day of response; however, enumerations on day 5 indicated an increased response in males exposed to the two lowest concentrations of TCE. On an AFC/10^6 cell basis, there was an increase of 50 and 54% in the 0.02 and 0.2 mg/ml-exposed males, while on an AFC/total spleen basis, there was a significant increase in only the 0.2 mg/ml-exposed males. Spleen size of day 5 males was significantly increased in the 0.02 mg/ml mice and decreased in the 2.0 mg/ml mice. Female mice exposed to the highest concentrations of TCE demonstrated a 40% increase in AFC/10^6 cells on the peak day of response; however, no change from control was noted on an AFC/spleen basis. No alteration on the AFC response was observed in females evaluated on day 5.

Hemagglutination titers were significantly depressed in a dose-dependent manner in both sexes when exposed to the two highest concentrations of TCE (Table 4). When the $\log_2$ titers presented in the data table were converted back to reciprocal antibody dilutions, there were 47% and 59% decreases in males and 40% and 45% decreases in females exposed to 0.2 and 2.0 mg/ml, respectively.

The responsiveness of spleen lymphocytes from male mice to the B cell mitogen LPS was unaltered from control (Table 5). Spleen cells from female mice exposed to the highest concentration of TCE demonstrated a significantly decreased response to optimal and sub-optimal concentrations of LPS (Table 6). This decreased response in the females was 27% less than control at the optimal concentration of LPS (5 μg) and 30% less at the sub-optimal concentration (20 μg). In the absence of mitogen there

TABLE 3
Humoral Immune Response to sRBC in CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	Spleen Wt. (mg)	AFC/Spleen (x 10^{-5})	AFC/10^6
Day 4		MALES	
Deionized water	170 ± 14	1.43 ± 0.15	1812 ± 129
0.02 mg/ml	200 ± 29	1.67 ± 0.20	1650 ± 199
0.2 mg/ml	172 ± 17	1.34 ± 0.23	1359 ± 191
2.0 mg/ml	132 ± 20	1.04 ± 0.20	1400 ± 209
		FEMALES	
Deionized water	177 ± 10	2.34 ± 0.24	1944 ± 156
0.02 mg/ml	185 ± 17	2.24 ± 0.45	1747 ± 270
0.2 mg/ml	159 ± 15	2.39 ± 0.28	2238 ± 154
2.0 mg/ml	152 ± 12	2.76 ± 0.34	2730 ± 194*
Day 5		MALES	
Deionized water	166 ± 6	0.65 ± 0.07	684 ± 78
0.02 mg/ml	206 ± 19*	1.04 ± 0.20	1023 ± 145*
0.2 mg/ml	189 ± 12	1.29 ± 0.20*	1052 ± 117*
2.0 mg/ml	131 ± 6*	0.74 ± 0.16	900 ± 133
		FEMALES	
Deionized water	156 ± 6	1.34 ± 0.12	1406 ± 100
0.02 mg/ml	179 ± 21	1.52 ± 0.27	1376 ± 134
0.2 mg/ml	139 ± 12	1.53 ± 0.27	1498 ± 163
2.0 mg/ml	150 ± 14	1.13 ± 0.24	1063 ± 152

[a]Values represent mean ± SE derived from 12 mice in the deionized water groups and 8 mice in the other groups.
*Significantly different from control at $p < 0.05$.

TABLE 4
Hemagglutination Titers in CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	Log_2 Titer Males	Females
Deionized Water	9.887 ± 0.164	10.061 ± 0.094
0.02 mg/ml	9.197 ± 0.239	9.760 ± 0.157
0.2 mg/ml	8.965 ± 0.341*	9.322 ± 0.242*
2.0 mg/ml	8.589 ± 0.358*	9.197 ± 0.364*

[a]Values represent mean ± SE derived from 23 mice in the deionized water group and 14-16 mice in the other groups.
*Significantly different from control at $p < 0.05$.

TABLE 5
Lymphocyte Responsiveness to Con A and LPS Using Spleens from Male CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	cpm/5 x 10^5 cells Con A	LPS
	1 µg/5 x 10^5 cells	1 µg/5 x 10^5 cells
Deionized water	105701 ± 7186	41662 ± 3470
0.02 mg/ml	94998 ± 11278	34184 ± 4909
0.2 mg/ml	121662 ± 7785	34627 ± 3560
2.0 mg/ml	92093 ± 5290	41092 ± 2971
	5 µg/5 x 10^5 cells	5 µg/5 x 10^5 cells
Deionized water	173150 ± 3264	41116 ± 3810
0.02 mg/ml	162262 ± 6185	39886 ± 6590
0.2 mg/ml	186082 ± 6816	38582 ± 4466
2.0 mg/ml	187989 ± 9403	40710 ± 3174
	10 µg/5 x 10^5 cells	20 µg/5 x 10^5 cells
Deionized water	61803 ± 8662	36123 ± 3022
0.02 mg/ml	57078 ± 11688	31081 ± 5737
0.2 mg/ml	77669 ± 11895	33738 ± 3084
2.0 mg/ml	57685 ± 7206	35776 ± 3390

[a]Values represent mean cpm's ± SE derived from 25 mice in the deionized water groups and 15-16 mice in the other groups.

TABLE 6
Lymphocyte Responsiveness to Con A and LPS Using Spleens from Female CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	cpm/5 x 10^5 cells Con A	LPS
	1 µg/5 x 10^5 cells	1 µg/5 x 10^5 cells
Deionized water	90704 ± 6895	51416 ± 3360
0.02 mg/ml	106682 ± 10444	51940 ± 3717
0.2 mg/ml	112900 ± 12162	55427 ± 5659
2.0 mg/ml	95991 ± 12884	39104 ± 4316
	5 µg/5 x 10^5 cells	5 µg/5 x 10^5 cells
Deionized water	153502 ± 4342	57878 ± 4061
0.02 mg/ml	164325 ± 3371	57055 ± 5008
0.2 mg/ml	158651 ± 5020	60425 ± 5892
2.0 mg/ml	138076 ± 4122*	42196 ± 4012*
	10 µg/5 x 10^5 cells	20 µg/5 x 10^5 cells
Deionized water	19302 ± 2026	50912 ± 3908
0.02 mg/ml	22115 ± 2152	47249 ± 5272
0.2 mg/ml	38156 ± 6187*	56645 ± 6526
2.0 mg/ml	26639 ± 2287	35517 ± 3620*

[a]Values represent mean cpm's ± SE derived from 25 mice in the deionized water groups and 15-16 mice in the other groups.
*Significantly different from control at $p < 0.05$.

was a slight increase in background DNA synthesis in female mice exposed to 0.2 mg/ml of TCE (Table 7).

Cell-mediated immune status was evaluated by the delayed-type hypersensitivity (DTH) (Table 8) and popliteal lymph node proliferation (Table 9) responses to sRBC. Cell-mediated immune status was not altered in any of the groups exposed to TCE as measured by these assays. Spleen lymphocyte responsiveness to

TABLE 7
Lymphocyte Responsiveness in the Absence of Mitogen Using Spleens from CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	cpm/5 x 10^5 cells Males	Females
Deionized Water	12755 ± 750	11770 ± 931
0.02 mg/ml	14904 ± 887	12468 ± 1328
0.2 mg/ml	15901 ± 1178	15660 ± 1230*
2.0 mg/ml	14264 ± 1812	14764 ± 1248

[a]Values represent mean ± SE derived from 25 mice in the deionized water group and 15-16 mice in the other groups.
*Significantly different from control at $p < 0.05$

TABLE 8
DTH Response to sRBC in CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	STIMULATION INDEX Males	Females
Deionized Water	3.54 ± 0.45	5.63 ± 0.64
0.02 mg/ml	3.42 ± 0.72	6.57 ± 0.88
0.2 mg/ml	3.98 ± 0.83	6.49 ± 0.45
2.0 mg/ml	4.01 ± 0.55	5.48 ± 0.67

[a]Values represent mean ± SE derived from 20 mice in the deionized water groups and 12-16 mice in the other groups. The unsensitized values was 1.92 for males and 2.01 for females.

TABLE 9
Popliteal Lymph Node Proliferation in CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	STIMULATION INDEX Males	Females
Deionized Water	11.7 ± 2.8	6.1 ± 1.1
0.02 mg/ml	10.6 ± 2.2	10.7 ± 2.3
0.2 mg/ml	8.4 ± 1.6	12.5 ± 2.6
2.0 mg/ml	12.7 ± 3.6	16.6 ± 2.5

[a]Values represent mean ± SE derived from 7-8 mice per group.

the T cell mitogen, Concanavalin A (Con A), was also evaluated (Tables 5 and 6). There were some alterations in the ability to respond in the females, but these did not appear to be dose-dependent.

The functional activity of the fixed macrophages of the reticuloendothelial system (RES) were evaluated by measuring the vascular clearance and organ distribution of chromated sRBC. The functional activity of male mice was unaltered (Table 10). The females (Table 11) exposed to 0.2 mg/ml demonstrated a 33% decrease in vascular clearance and a 37% increase in the percent of radiolabel taken up by the spleen. The females exposed to the highest concentration of TCE, 2.0 mg/ml, demonstrated a 33% increase in vascular clearance, and, on a specific activity basis only, a 17% increase of radiolabel in the liver and a 54% decrease in the spleen.

The numbers and activities of thioglycolate-recruited PECs for both sexes are summarized in Table 12. PECs from male mice exposed to the highest concentration of TCE have a decreased phagocytic ability, as demonstrated by the lowered amount of chromated sRBC measured in these cells over a 20 to 45 min period. No alterations were observed in the females.

TABLE 10
Functional Activity of the RES in Male CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	Body Weight (g)	Phagocytic Index	Organ	% Uptake[b]	cpm/mg[c]
Deionized water	39.8 ± 0.8	0.10 ± 0.01	Liver	52.6 ± 2.0	194 ± 9
			Spleen	7.2 ± 1.0	423 ± 78
			Lungs	0.8 ± 0.1	17 ± 3
			Thymus	.005 ± .001	4 ± 1
			Kidneys	1.8 ± 0.2	20 ± 2
0.02 mg/ml	39.6 ± 0.8	0.09 ± 0.01	Liver	60.2 ± 3.3	214 ± 15
			Spleen	6.2 ± 1.4	351 ± 105
			Lungs	1.2 ± 0.3	33 ± 9
			Thymus	.005 ± .002	4 ± 1
			Kidneys	1.9 ± 0.4	21 ± 5
0.2 mg/ml	35.8 ± 1.7*	0.09 ± 0.01	Liver	59.6 ± 5.1	206 ± 12
			Spleen	8.2 ± 1.5	306 ± 73
			Lungs	1.2 ± 0.3	28 ± 9
			Thymus	.006 ± .001	6 ± 2
			Kidneys	2.2 ± 0.5	26 ± 7
2.0 mg/ml	35.1 ± 1.9*	0.13 ± 0.05	Liver	56.5 ± 3.9	186 ± 8
			Spleen	6.6 ± 1.3	401 ± 127
			Lungs	0.8 ± 0.2	18 ± 4
			Thymus	.005 ± .001	7 ± 2
			Kidneys	1.3 ± 0.4	15 ± 5

[a]Values represent mean ± SE derived from 14 mice in the deionized water group and 7-8 mice in the other groups.
[b]% Uptake = (cpm in organ/cpm injected) x 100
[c]cpm/mg = cpm/mg in organ
*Significantly different from control at $p < 0.05$

TABLE 11
Functional Activity of the RES in Female CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	Body Weight (g)	Phagocytic Index	Organ	% Uptake[b]	cpm/mg[c]
Deionized water	31.0 ± 0.7	0.09 ± 0.01	Liver	53.6 ± 1.9	213 ± 9
			Spleen	8.6 ± 1.0	476 ± 67
			Lungs	1.2 ± 0.2	31 ± 4
			Thymus	.008 ± .001	6 ± 2
			Kidneys	2.9 ± 0.2	42 ± 3
0.02 mg/ml	30.3 ± 0.9	0.09 ± 0.01	Liver	48.9 ± 3.6	177 ± 12
			Spleen	6.9 ± 0.7	398 ± 84
			Lungs	1.4 ± 0.2	40 ± 5
			Thymus	.006 ± .001	13 ± 5
			Kidneys	2.6 ± 0.5	36 ± 6
0.2 mg/ml	29.5 ± 0.9	0.06 ± 0.01*	Liver	52.2 ± 2.7	217 ± 10
			Spleen	11.8 ± 1.6*	583 ± 101
			Lungs	1.4 ± 0.2	39 ± 7
			Thymus	.009 ± .001	5 ± 1
			Kidneys	2.7 ± 0.1	43 ± 3
2.0 mg/ml	30.0 ± 0.3	0.12 ± 0.01*	Liver	60.4 ± 2.4	250 ± 14*
			Spleen	5.6 ± 0.8	219 ± 27*
			Lungs	0.8 ± 0.1	22 ± 5
			Thymus	.011 ± .002	2 ± 1
			Kidneys	2.3 ± 0.2	31 ± 2

[a]Values represent mean ± SE derived from 16 mice in the deionized water group and 8 mice in the other groups.
[b]% Uptake = (cpm in organ/cpm injected) x 100
[c]cpm/mg = cpm/mg in organ
*Significantly different from control at $p < 0.05$

TABLE 12
Number and Activities of Thioglycolate-Recruited PEC in CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	Recruitable PEC ($\times 10^{-7}$)	Adherent PEC ($\times 10^{-5}$)	Chemotaxis (cells/field)
	MALES		
Deionized water	1.32 ± 0.27	2.15 ± 0.09	234 ± 34
0.02 mg/ml	1.62 ± 0.29	2.18 ± 0.24	426 ± 74
0.2 mg/ml	1.26 ± 0.20	2.85 ± 0.33*	451 ± 75
2.0 mg/ml	0.81 ± 0.25	1.80 ± 0.14	349 ± 138
	FEMALES		
Deionized water	2.38 ± 0.42	2.78 ± 0.38	259 ± 44
0.02 mg/ml	1.48 ± 0.16	2.52 ± 0.22	324 ± 56
0.2 mg/ml	1.45 ± 0.23	2.15 ± 0.29	502 ± 111
2.0 mg/ml	1.83 ± 0.14	3.45 ± 0.46	408 ± 96

	PHAGOCYTOSIS (CPM)			
Exposure Group	10 min	20 min	30 min	45 min
	MALES			
Deionized water	456 ± 79	660 ± 88	744 ± 120	893 ± 185
0.02 mg/ml	384 ± 86	424 ± 68	490 ± 113	536 ± 88
0.2 mg/ml	501 ± 97	725 ± 159	790 ± 201	1252 ± 298
2.0 mg/ml	270 ± 24	293 ± 52*	338 ± 56*	404 ± 71*
	FEMALES			
Deionized Water	709 ± 129	1002 ± 189	1182 ± 310	927 ± 153
0.02 mg/ml	558 ± 130	664 ± 212	660 ± 250	728 ± 301
0.2 mg/ml	286 ± 39	478 ± 95	550 ± 181	646 ± 206
2.0 mg/ml	422 ± 141	862 ± 219	823 ± 195	1570 ± 415

[a]Values represent mean ± SE derived from 9-12 mice per group.
*Significantly different from control at $p < 0.05$

TABLE 13
Bone Marrow DNA Synthesis in CD-1 Mice Exposed to TCE in the Drinking Water for 90 Days[a]

Exposure Group	cpm/6 x 10^5 cells			
	MALES		FEMALES	
	60 min	120 min	60 min	120 min
Deionized water	19913 ± 1192	33095 ± 2189	20508 ± 999	35700 ± 1965
0.02 mg/ml	20313 ± 1230	30982 ± 1905	15752 ± 994*	29582 ± 2257
0.2 mg/ml	21504 ± 1479	35516 ± 2936	20365 ± 1153	35222 ± 1925
2.0 mg/ml	25041 ± 3059	32159 ± 2431	16255 ± 1123*	34700 ± 1592

[a]Values represent mean cpm's ± SE derived from 12 mice in the deionized water group and 8 mice in the other groups.
*Significantly different from control at $p < 0.05$

The ability of bone marrow cells from mice exposed to TCE for 90 days to incorporate ^{125}I-IUdR was essentially unaffected (Table 13). There were slight decreases in DNA synthesis following 60 min of incubation with ^{125}I-IUdR in some of the females; however, this decrease was not seen in the 120 min incubation period.

DISCUSSION

The objective of the study presented in this manuscript was to evaluate the effects of TCE on the immune system of male and female random-bred CD-1 mice. Recent investigations in our laboratory have been aimed at incorporation of immune function assays into more standard toxicological evaluations. From the immunological viewpoint, the inbred or F_1 hybrid mouse is the animal of choice, whereas the random-bred rat would be more appropriate for a general toxicological study. In these studies,

we have chosen to use the CD-1 outbred mouse. Our subchronic studies have utilized both sexes, as should be done in a comprehensive evaluation of any chemical.

Even though no immunological alterations were observed in male mice following 14 days of exposure, a more detailed 90-day evaluation, utilizing higher dose levels, revealed effects in both males and females.

Serum antibody to sRBC was depressed in TCE-treated mice as evaluated by measuring hemagglutination titers (Table 4). However, when measuring AFC to sRBC, there was an enhancement of the response is some groups (Table 3). The opposite results of the two assays could be due to the fact that only IgM antibody is measured in the plaque assay, whereas mostly IgG is detected using the agglutination methods. Also, the plaque assay is enumerating individual antibody forming cells of the spleen, while the agglutination assay is measuring levels of antibody in the serum. These results indicate that is may be desirable to use more than one assay to evaluate the humoral immune response due to the fact that the different assays measure different parameters.

Spleen lymphocyte responsiveness to the B cell mitogen, LPS, was unaltered in males, but significantly decreased in females exposed to the highest concentration of TCE (Tables 5 and 6). This depressed response in the females occurred not only at the optimum concentration of LPS, but also at the sub-optimal concentration, which indicates that the depression could not be overcome by the addition of more mitogen. The humoral immune response in females appeared to be more sensitive to TCE's effects than males, as measured by the hemagglutination assay (13% depressed in females as opposed to 9% depressed in males). This would correlate with the higher sensitivity to the effects of TCE in the females over males in the assay measuring lymphocyte responsiveness to LPS.

Cell-mediated immune status was unaltered in either sex of mice, even though the females exposed to the highest concentration of TCE did demonstrate a slightly decreased response to the optimum concentration of Con A. This decreased response, however, was not seen at the lower or higher concentrations of Con A, and therefore appears to indicate that the functional ability of these cells is essentially intact.

The fixed macrophage system, as evaluated by the functional ability of the RES, was unaltered in the males. In the females exposed to 2.0 mg/ml of TCE there was an increased clearance of sRBC and it appeared that this was due to an increase in uptake of the sRBC into the liver. The decreased uptake into the spleen in this group of mice was probably due to the decreased availability of particles as a result of increased hepatic uptake. When PECs were assessed for macrophage function, the PECs from females were not affected, but the males exposed to the highest level of TCE demonstrated a significantly depressed ability to phagocytize sRBC. As the time course of this experiment was not carried out through a plateau point, it can only be concluded that the rate of phagocytosis was affected and not necessarily the total phagocytic capacity of these cells.

In conclusion, it appears that there are sex differences in the ability of TCE to alter immune function, and these differences can only be seen when certain assays are employed. It is important to use a variety of assays assessing immune responsiveness to be certain that an effect by a chemical is detected.

ACKNOWLEDGEMENTS

This work was supported by grants from the Environmental Protection Agency (R806481010) and the National Institute of Environmental Health Sciences (1T32ES07087).

REFERENCES

1. K.L. White, Jr., V.M. Sanders, D.W. Barnes, G.M. Shopp, Jr., L.E. Sain, J.F. Borzelleca, and A.E. Munson, Toxicology of 1,1,2-Trichloroethane in the mouse, Drug Chem. Toxicol., _____ (1985).

2. K.L. White, Jr., V.M. Sanders, D.W. Barnes, L.E. Sain, G.M. Shopp, Jr., R.A. Carchman, J.F. Borzelleca, and A.E. Munson, Immunotoxicological investigations in the mouse. General approach and methods, Drug Chem. Toxicol., _____ (1985).

3. J.P. Sall, Duncan Procedure, in SAS User's Guide, 1979 Edition, J.T. Helwig and K.A. Council, eds., SAS Institute, Inc., Raleigh, NC, 1979, pp. 191-194.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.